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# 32

# NITRATE RESPIRATION AND NITRIFICATION IN ESTUARINE SEDIMENTS

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ABSTRACT - Seasonal data and depth profiles using $^{15}N$-labelled $NO_3^-$ show that denitrification (77-90 % of $NO_3^-$ respired) rather than $NO_3^-$ dissimilation to $NH_4^+$ was the principal route of nitrate reduction in Kingoodie Bay sediments. Populations of both groups of $NO_3^-$ reducing bacteria were highest in the 0-20 mm horizon in those sediments where highest rates of $NO_3^-$ respiration were recorded (28.56 $\mu g$ N.$d^{-1}$ dry wt. sediment $^{-1}$). Autotrophic nitrification rates shared a marked seasonality with highest rates (0.92 $\mu g$ N.$d^{-1}$.g dry wt. sediment $^{-1}$) occurring during the summer. Maximum populations of autotrophic nitrifying bacteria were also found in the 0-20 mm sediment horizon and these data indicate that both processus occur simultaneously in the oxidised surface sediments.

*Key words :* denitrification, nitrification, nitrate reduction to ammonia.

RÉSUMÉ - Les données saisonnières et les profils verticaux obtenus en utilisant $^{15}N.NO_3$, montrent que la dénitrification est la voie principale de la réduction des nitrates dans les sédiments de la baie de Kingoodie. Ce processus représente 77 à 90% des nitrates respirés et est plus important que la transformation des nitrates en ammoniaque. Les deux groupes de bactéries pouvant réduire les nitrates sont les plus développés à la surface du sédiment (0-20 mm) où les taux de respiration des nitrates sont aussi les plus élevés (28.56 $\mu g$N/jour/g de sédiment sec). D'autre part, les taux de nitrification autotrophe présentent un rythme saisonnier marqué avec des maxima en été (0.92 $\mu g$ N/jour/g de poids sec). Les populations de bactéries nitrifiantes autotrophes sont également maximales à la surface du sédiment (0-20 mm).

*Mots clés :* dénitrification, nitrification, réduction des nitrates en ammoniaque.

## INTRODUCTION

Estuarine and inshore marine surface sediments (0-50 mm depth) are environments where sharp discontinuities in dissolved oxygen tension occur over extremely small vertical distances or within microniches (Jorgensen, 1977 ; Billen, 1982). In such habitats micro-organisms of widely differing physiological types can co-exist and be metabolically active. In anoxic sediments the respiratory reduction of nitrate is an alternative to aerobic metabolism and is energetically superior to fermentation. The end product(s) of reduction depend upon the micro-organism and growth conditions and may be either nitrite ($NO_2^-$), ammonia ($NH_4^+$), nitrous oxide ($N_2O$) or gaseous nitrogen ($N_2$). When the end-products are gaseous the process is more correctly termed denitrification, whereas if they are $NO_2^-$ of $NH_4^+$ the pathway is one of nitrate dissimilation (Herbert *et al.*, 1980; Macfarlane and Herbert, 1982).

The nitrogen intermediates involved in the anaerobic dissimilation of $NO_3^-$ to $NH_4^+$ are the reverse of those involved in nitrification, the aerobic oxidation of $NH_4^+$ to $NO_3^-$ by nitrifying bacteria (Herbert, 1982). The objective of this present investigation was to determine the relationship between populations of nitrate respiring bacteria and nitrifying bacteria in estuarine sediments in respect of the physico-chemical parameters which modulate their activities.

## MATERIALS AND METHODS

### *Sampling sites*

The main sampling area was in a tidal region of mud flats at Kingoodie Bay in the River Tay estuary, west of Dundee and 16 Km from the river mouth. Samples from the top 50 mm sediment were taken at low water using a sterile 50 mm diameter × 150 mm long perspex corer. All samples were processed within 1 hour of collection and aseptically sectioned as described by Macfarlane and Herbert (1982).

### *Physical characteristics of Kingoodie Bay sediments*

Measurements of $E_h$, temperature and dissolved $O_2$ tension were made *in situ* in 10 mm increments according to the methods described by Macfarlane and Herbert (1982).

### *Enumeration of nitrate respiring and nitrifying bacteria*

Population densities of nitrate respiring bacteria and nitrifying bacteria were determined according to the methods described by Macfarlane and Herbert (1984a).

### *Determination of nitrate respiration rates using* $^{15}NO_3^-$

Nitrate respiration rates were determined according to the method of Macfarlane and Herbert (1984a).

### *Determination of nitrification rates*

Nitrification rates were determined according to the methods of Billen (1975).

### *Inorganic nitrogen analysis*

Ammonia, $NO_3^-$ and $NO_2^-$ concentrations were determined according to the methods described by Macfarlane and Herbert (1984a).

### *Chemicals*

N-serve (2-chloro-6 (trichloromethyl) pyridine) was a gift from the Dow Chemical Company and $Na^{15}NO_3$ was obtained from BOC Prochem, London, UK. All other chemicals used were of 'Analar' grade and obtained from B.D.H., Poole, UK.

## RESULTS

The surface sediments in Kingoodie Bay are composed of fine sands overlain with silt and are highly reduced within a few mm of the surface. The principal physico-chemical characteristics of the sediments are summarised in Table 1. The surface sediments are more oxidised during winter due to a combination of reduced microbial activity and increased resuspension and oxygenation of the sediments. With the exception of the 0-10 mm horizon total $NH_4^+$ concentrations are substantially greater than those of $NO_3^-$ and $NO_2^-$ and increase with depth. In contract $NO_3^-$ concentrations show a reverse pattern with highest concentrations at the surface.

| Depth (mm) | Eh (mV) | $O_2$ conc$^n$ (mg. $l^{-1}$) | $NH_4^+$ * | $NO_2^-$ * | $NO_3^-$ * |
|---|---|---|---|---|---|
| 0-10 | +320 | 8.7 | 50 | 19 | 95 |
| 10-20 | +200 | 1-6 | 460 | 25 | 85 |
| 20-30 | +50 | N.D | 510 | N.D. | 54 |
| 30-40 | -103 | N.D. | 516 | N.D. | N.D. |
| 40-50 | -190 | N.D. | 530 | N.D. | N.D. |

* n mol ml $^{-1}$ interstitial water
N.D. : not detected

Table 1 : Typical physico-chemical profiles in Kingoodie Bay sediments during July 1982. Results are mean values of three samples.

| Depth (mm) | | Month | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sept | Oct |
| 0-10 | $NH_4^+$ oxidisers | 8 | 14 | 37 | 52 | 44 | 8 | 6 | 94 | 18 | 14 | 11 |
| | $NO_2^-$ oxidisers | 7 | 12 | 18 | 26 | 40 | 17 | 15 | 90 | 9 | 7 | 10 |
| 20-30 | $NH_4^+$ oxidisers | 2 | 5 | 6 | 8 | 13 | 12 | 10 | 88 | 10 | 22 | 1 |
| | $NO_2^-$ oxidisers | 2 | 6 | 8 | 13 | 19 | 6 | 6 | 120 | 19 | 12 | 6 |
| 40-50 | $NH_4^+$ oxidisers | 1 | 2 | 2 | 2 | 8 | 5 | 1 | 24 | 4 | 4 | 6 |
| | $NO_2^-$ oxidisers | 0.5 | 1 | 1 | 1 | 3 | 10 | 2 | 14 | 2 | 2 | 1 |

Table 2 : Population densities of autotrophic $NH_4^+$ oxidising and $NO_2^-$ oxidising bacteria in Kingoodie Bay sediments. December 1981 to October 1982. Cell number expressed as MPN x $10^3$ viable cells. g dry wt. sediment$^{-1}$.

## *Seasonal and spatial distribution of $NO_3^-$ respiring and nitrifying bacteria*

Data presented in Table 2 show that the highest cell densities of $NH_4^+$ and $NO_2^-$ oxidising bacteria were present in the 0-20 mm depth horizon and below this depth there was a rapid decline in cell numbers. A marked seasonality of autotrophic nitrifying bacteria was recorded with cell population maxima being recorded in March/April and July.

Determination of population densities of nitrate respiring bacteria in Kingoodie Bay sediment showed that those bacteria respiring $NO_3^-$ to $NH_4^+$ were always numerically dominant (up to a factor of $10^2$) compared with those denitrifying $NO_3^-$ to gaseous products (Table 3). Data in Table 3 show that the nitrate dissimilating bacteria in Kingoodie Bay sediments apparently migrate on a seasonal basis. In autumn and winter maximum cell populations were present in the 10-20 depth horizon but in the spring and early summer there was an apparent migration to the 0-10 mm horizon. These data are consistent with the recorded seasonal changes in Eh profiles of Kingoodie Bay sediments which are more reduced in summer than in winter (Macfarlane and Herbert, 1984a).

## *Nitrification rates in Kingoodie Bay sediments*

Maximum rates of nitrification, as measured by the $^{14}C$ bicarbonate dark uptake method, were recorded in the 0-10 mm horizon and rapidly decreased with depth (Table 4). No significant activity was observed at depths greater than 30 mm and are in agreement with the bacterial count data (Table 2). Maximum nitrification rates (0.92 $\mu g$ N.d$^{-1}$. g dry wt.

sediment $^{-1}$) were recorded 0-10 mm depth horizon during the summer when the sediments were warmest (19°C) and lowest during the winter (mean temperature 4.5°C). Little correlation was observed between population densities of nitrifying bacteria (Table 2) and recorded nitrification rates (Table 4) indicating that temperature exerted a more profound effect on nitrifying activity than cell numbers. The addition of 5 mg N-serve. $l^{-1}$, a potent inhibitor of autotrophic $NH_4^+$ oxidising bacteria (Campbell and Aleem, 1965), totally inhibited nitrification indicating that autotrophic $NH_4^+$ oxidation rather than heterotrophic nitrification was the principal process occurring in these sediments.

| Depth (mm) | | Month | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sept | Oct |
| 0-10 | $NO_3^-$ $NH_4^+$ | 4.43 | 3.12 | 21.1 | 39.3 | 36.2 | 29.5 | 23.7 | 6.77 | 1.24 | 0.95 | 0.86 |
| | $NO_3^-$ $N_2$ | 0.06 | 0.31 | 0.25 | 0.22 | 0.41 | 0.38 | 0.46 | 0.41 | 0.37 | 0.22 | 0.20 |
| 10-20 | $NO_3^-$ $NH_4^+$ | 6.31 | 7.14 | 7.23 | 5.01 | 4.62 | 5.04 | 6.31 | 18.0 | 11.2 | 9.12 | 8.71 |
| | $NO_3^-$ $N_2$ | 0.04 | 0.17 | 0.16 | 0.15 | 0.10 | 0.09 | 0.10 | 0.12 | 0.11 | 0.10 | 0.03 |
| 20-30 | $NO_3^-$ $NH_4^+$ | 0.83 | 0.96 | 3.9 | 1.4 | 1.0 | 0.97 | 1.12 | 1.36 | 0.26 | 0.21 | 0.39 |
| | $NO_3^-$ $N_2$ | 0.04 | 0.05 | 0.21 | 0.10 | 0.09 | 0.10 | 0.14 | 0.12 | 0.11 | 0.10 | 0.02 |
| 30-40 | $NO_3^-$ $NH_4^+$ | 0.50 | 0.51 | 0.56 | 3.9 | 0.58 | 0.47 | 0.61 | 0.57 | 0.58 | 0.1 | 0.08 |
| | $NO_3^-$ $N_2$ | 0.06 | 0.05 | 0.04 | 0.04 | 0.06 | 0.12 | 0.11 | 0.09 | 0.10 | 0.07 | 0.04 |
| 40-50 | $NO_3^-$ $NH_4^+$ | 0.25 | 0.27 | 0.19 | 0.22 | 0.26 | 0.37 | 0.49 | 0.41 | 0.26 | 0.14 | 0.11 |
| | $NO_3^-$ $N_2$ | 0.03 | 0.01 | 0.02 | 0.03 | 0.04 | 0.03 | 0.05 | 0.04 | 0.03 | 0.02 | 0.02 |

Table 3 : Population densities of nitrate dissimilating and denitrifying bacteria in Kingoodie Bay sediments. December 1981 to October 1982. Cell numbers expressed as MPN x $10^6$ viable cells. dry g wt sediment $^{-1}$.

| Depth (mm) | Month | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct |
| 0-10 | 0.13 | 0.12 | 0.18 | 0.29 | 0.665 | 0.67 | 0.92 | 0.81 | 0.69 | 0.54 | 0.63 |
| 10-20 | 0.03 | 0.07 | 0.12 | 0.17 | 0.20 | 0.57 | 0.66 | 0.64 | 0.17 | 0.22 | 0.16 |
| 20-30 | 0.005 | 0.01 | 0.01 | 0.04 | 0.06 | 0.22 | 0.31 | 0.37 | 0.24 | 0.16 | 0.04 |

Table 4 : Nitrifying activity in Kingoodie Bay sediments expressed as $\mu g$ N.d $^{-1}$ g dry wt. sediment $^{-1}$, December 1981 to October 1982.

### *Nitrate respiration in Kingoodie Bay sediments*

Maximum rates of nitrate respiration were recorded during the summer months and in the 10-20 mm depth horizon (Table 5). Although the rates of nitrate respiration were lower in the 0-10 mm horizon considerable activity was still recorded (82% of that recorded at 10-20 mm depth in July 1982). Data in Table 5 show unequivocally that denitrification is the principal process of nitrate respiration in Kingoodie Bay sediments and are in agreement with those of Sorensen (1978) for Danish coastal marine sediments and of Koike and Hattori (1978) for marine sediments in Japan. Whilst $NO_3^-$ dissimilation to $NH_4^+$ is the minor route of nitrate respiration in Kingoodie Bay sediments it is not an inconsequential process and data in Table 5 show that with increasing depth an increasing proportion of $NO_3^-$ was reduced to $NH_4^+$ although the total quantities of $NO_3^-$ respired was substantially less than in the surface sediment.

| Depth (mm) | November 1981 | | | July 1982 | | |
|---|---|---|---|---|---|---|
| | $N_2$ | $NH_4^+$ | Total | $N_2$ | $NH_4^+$ | Total |
| 0-10 | 6.2 | 1.1 | 7.3 | 18.9 | 4.6 | 23.5 |
| 10-20 | 9.8 | 2.1 | 11.9 | 24.4 | 4.1 | 28.5 |
| 20-30 | 5.2 | 6.1 | 11.3 | 11.6 | 3.5 | 15.1 |
| 30-40 | 2.2 | 1.3 | 3.5 | 4.7 | 2.4 | 7.1 |
| 40-50 | 2.3 | 1.07 | 3.3 | 4.4 | 1.7 | 6.1 |

Table 5 : Rates of denitrification and nitrate dissimilation to $NH_4^+$ in Kingoodie Bay sediments as determined using $^{15}N$-labelled nitrate. Rates are expressed as $\mu g\ N.d^{-1}\ g$ dry wt. sediment $^{-1}$ and are mean values of 3 replicates.

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